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(54) Title: ASSAYS FOR AGONISTS, AGONISTS AND INVERSE AGONISTS OF MELANIN CONCENTRATING HORMONE (MCH) BINDING TO THE SOMATOSTATIN-LIKE RECEPTOR (SLC-1)			
(57) Abstract <p>The present invention is directed to assays that can be used to screen for compounds that act as agonists or antagonists of melanin concentrating hormone (MCH). The assays are based upon the binding of MCH to the SLC-1 receptor.</p>			

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ASSAYS FOR AGONISTS, AGONISTS AND INVERSE AGONISTS OF MELANIN CONCENTRATING HORMONE (MCH) BINDING TO THE SOMATOSTATIN-LIKE RECEPTOR (SLC-1)

Field of the Invention

The present invention is directed to assay methods that can be used to determine whether a test compound can be used to modulate the binding of MCH to the SLC-1 receptor. Compounds identified as being effective modulators have potential use as therapeutic agents in treating obesity and eating disorders.

Background of the Invention

A. Melanin Concentrating Hormone

Melanin concentrating hormone (MCH) is a cyclic peptide that was first isolated from fish over 15 years ago. In mammals, MCH gene expression is localized to the ventral aspect of the zona inserta and the lateral hypothalamic area (Breton, *et al.*, *Mol. Cell. Neurosci.* 4:271-283 (1993)). The latter region of the brain is associated with the control of behaviors such as eating and drinking, with arousal and with motor activity (Baker, *Trends Endocrinol. Metab.* 5:120-126 (1994)). Although the biological activity of MCH in mammals has not been fully defined, recent work has indicated that it promotes eating and weight gain (U.S. 5,849,708). Thus, MCH and its agonists have been proposed as a treatment for anorexia nervosa and weight loss due to AIDS, renal disease, or chemotherapy. Similarly, antagonists of MCH can be used as a treatment for obesity and other disorders characterized by compulsive eating and excessive body weight.

Although MCH has been known for over two decades, its specific receptor has not been structurally characterized and cloned. This has limited the ability to search for therapeutic agents that act by mimicking or inhibiting MCH.

B. G Protein-Coupled Receptors

G protein coupled receptors (GPCRs) constitute a family of proteins sharing a common structural organization characterized by an extracellular N-terminal end, seven hydrophobic alpha helices putatively constituting transmembrane domains and an intracellular C-terminal domain. GPCRs bind a wide variety of ligands that trigger intracellular signals

through the activation of transducing G proteins (Caron, *et al.*, *Rec. Prog. Horm. Res.* 48:277-290 (1993); Freedman, *et al.*, *Rec. Prog. Horm. Res.* 51:319-353 (1996)).

More than 300 GPCRs have been cloned thus far and it is generally assumed that there exist well over 1,000 such receptors. Roughly 50-60% of all clinically relevant drugs act by modulating the functions of various GPCRs (Gudermann, *et al.*, *J. Mol. Med.* 73:51-63 (1995)). Many of the clinically relevant receptors are located in the central nervous system.

Among the GPCRs that have been identified and cloned is a gene that encodes a protein homologous to the receptors of the somatostatin family. Kolakowski called this receptor SLC-1 and described the structure of the gene as it exists in humans (*FEBS Lett.* 398:253-258, (1996)). A rat counterpart of SLC-1 was found to be essentially identical and was described by Lakaye, *et al.* (*Biochim. Biophys. Acta.* 1401:216-220 (1998)). Based upon the location of cells expressing SLC-1 mRNA, it was proposed that the receptor plays a role in functions such as emotion, memory and sensory perception. However, the endogenous ligand of this receptor has not previously been identified.

15 Summary of the Invention

The present invention is based upon the discovery that MCH serves as a ligand for the SLC-1 receptor. Recombinant cells expressing either rat or human SLC-1 can be used in conjunction with MCH in screening assays designed to identify agonists and antagonists. Thus, in its first aspect, the invention is directed to a method of assaying a test compound for 20 its ability to bind to the SLC-1 receptor. This is accomplished by incubating cells expressing the receptor gene with MCH and test compound. The extent to which the binding of MCH is displaced is then determined. Radioligand assays or enzyme-linked immunosorbent assays may be performed in which either MCH or the test compound is detectably labeled. Although any cell expressing SLC-1 may be used, a recombinant cell expressing a heterologous SLC-1 25 gene from either the rat or human is preferred. The term "heterologous" as used herein refers to any SLC-1 gene transfected into a cell, *i.e.*, the term refers to any non-endogenous SLC-1.

The invention is also encompasses methods of determining if a test compound is an agonist, antagonist, or inverse agonist of MCH binding based upon a functional assay. One way to carry out such assays is to incubate a cell expressing SLC-1 with the test compound

and to then determine whether intracellular adenyl cyclase activity or intracellular calcium concentration changes. Results should typically be compared with those obtained when incubations are performed in a similar manner but in the absence of test compound. In general, functional assays of this type will be performed in conjunction with binding assays of
5 the sort described above. The preferred cell for use in the assays is a recombinant cell that has been transformed with a heterologous SLC-1 gene. Test compounds that act as agonists should produce an increase or decrease in adenyl cyclase activity or increase in intracellular levels of calcium. Inverse agonists may reduce adenyl cyclase activity or intracellular calcium levels, particularly if assays are performed in the presence of a fixed amount of MCH.
10 Antagonists, should block the binding of MCH to receptor but not produce the opposite response in terms of adenyl cyclase activity or intracellular calcium that is the hallmark of an inverse agonist.

Detailed Description of the Invention

The present invention is directed to assays that can be used to screen compounds for
15 their ability to modulate the binding of MCH to the SLC-1 receptor. Any form of MCH that has been reported may be used, but the preferred peptide is 19 amino acids in length and has the sequence: Asp-Phe-Asp-Met-Leu-Arg-Cys-Met-Leu-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Gln-Val (SEQ ID NO:1). The peptide assumes a cyclic conformation as the result of a disulfide between the two cysteines. This peptide may be obtained commercially (Sigma,
20 St. Louis, MO) or can be synthesized using standard methodology well known in the art. The peptide may be detectably labeled with radioisotopes such as ¹²⁵I or, alternatively, fluorescent or chemiluminescent labels can be incorporated. Also, the peptide can be joined to enzymes that are readily detectable such as horseradish peroxidase.

The SLC-1 receptor may be cloned from human cells using the procedure described by
25 Kolakowski, *et al.* (*FEBS Lett.* 398:253-258 (1996)) or from rat cells using the procedure described by Lakaye, *et al.* (*Biochim. Biophys. Acta.* 1401:216-220 (1998)). The Examples section provides a detailed description of a procedure that may be used in cloning SLC-1 which, is also referred to herein as clone 1-18. Once obtained, the SLC-1 sequence should be incorporated into an expression vector with a promoter active in mammalian cells (Sambrook,
30 *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press (1989)). Examples of promoters that may be used include that of the mouse metallothionein I gene

(Hamer, *et al.*, *J. Mol. Appl. Gen.* 1:273-288 (1982)); the immediate-early and TK promoter of herpes virus (Yao, *et al.*, *J. Virol.* 69:6249-6258 (1995); McKnight, *Cell* 31:355-365 (1982)); the SV 40 early promoter (Benoist, *et al.*, *Nature* 290:304-310 (1981)); and, the CMV promoter (Boshart, *et al.*, *Cell* 41:521-530 (1985)). Vectors may also include enhancers and other regulatory elements.

Once expression vectors have been constructed, they can be introduced into a mammalian cell line by methods such as calcium phosphate precipitation, microinjection, electroporation, liposomal transfer, viral transfer or particle mediated gene transfer. Although other mammalian cells may be used, HEK-293 cells have been found to give successful results and a procedure for expressing SLC-1 in these cells is described in the Examples section. Standard procedures for selecting cells and for assaying them for the expression of SLC-1 (*e.g.*, by Northern analysis) may be performed.

Once the MCH peptide and cells producing the SLC-1 receptor have been obtained, assays may be performed to determine whether test compounds have any effect on binding. A wide variety of different types of assays can be performed using standard methods well known in the art. For example, in radioligand binding assays, cells expressing SLC-1 are incubated with MCH and with a compound being tested for binding activity. The preferred source of SLC-1 is recombinantly transformed HEK-293 cells. Other cells may also be used provided they do not express other proteins that strongly bind MCH. This can easily be determined by performing binding assays on cells transformed with SLC-1 and comparing the results obtained with those obtained using their untransformed counterparts.

Assays may be performed using either intact cells or with membranes prepared from the cells (*see e.g.*, Wang, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90:10230-10234 (1993)). As suggested above, the membranes, or cells, are incubated with MCH and with a preparation of the compound being tested. After binding is complete, receptor is separated from the solution containing ligand and test compound, *e.g.*, by filtration, and the amount of binding that has occurred is determined. Preferably, the ligand used is detectably labeled with a radioisotope such as ¹²⁵I. However, if desired, other types of labels can also be used. Among the most commonly used fluorescent labeling compounds are fluorescein, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin *o*-phthaldehyde and fluorescamine. Useful

chemiluminescent compounds include luminol, isoluminol, theromatic of acridinium ester, imidazole, acridinium salt, and oxalate ester.

Nonspecific binding may be determined by carrying out the binding reaction in the presence of a large excess of unlabeled ligand. For example, labeled MCH may be incubated
5 with receptor and test compound in the presence of a thousandfold excess of unlabeled MCH. Nonspecific binding should be subtracted from total binding, *i.e.*, binding in the absence of unlabeled ligand, to arrive at the specific binding for each sample tested. Other steps such as washing, stirring, shaking, filtering and the like may be included in the assays as necessary. Typically, wash steps are included after the separation of membrane-bound ligand from ligand
10 remaining in solution and prior to quantitation of the amount of ligand bound, *e.g.*, by counting radioactive isotope. The specific binding obtained in the presence of test compound is compared with that obtained in the presence of labeled ligand alone to determine the extent to which the test compound has displaced receptor binding.

In performing binding assays, care must be taken to avoid artifacts which may make it
15 appear that a test compound is interacting with receptor when, in fact, binding is being inhibited by some other mechanism. For example, the compound being tested should be in a buffer which does not itself substantially inhibit the binding of MCH and should, preferably, be tested at several different concentrations. Preparations of test compound should also be examined for proteolytic activity and it is desirable that antiproteases be included in assays.
20 Finally, it is highly desirable that compounds identified as displacing the binding of MCH be reexamined in a concentration range sufficient to perform a Scatchard analysis on the results. This type of analysis is well known in the art and can be used for determining the affinity of a test compound for receptor (*see e.g.*, Ausubel, *et al.*, Current Protocols and Molecular Biology, 11.2.1-11.2.19 (1993); Laboratory Techniques in Biochemistry and Molecular Biology, Work, *et al.*, Ed. N.Y. (1978)). Computer programs may be used to help in the
25 analysis of results (*e.g.*, Munson, P., *Methods Enzymol.* 92:543-577 (1983)).

Depending upon their effect on the activity of the receptor, agents that inhibit the binding of MCH to receptor may be either agonists or antagonists. Activation of receptor may be monitored using a number of different methods. For example, adenyl cyclase assays may
30 be performed by growing cells in wells of a microtiter plate and then incubating the wells in

the presence or absence of test compound. cAMP may then be extracted in ethanol, lyophilized and resuspended in assay buffer. Assay of cAMP thus recovered can be carried out using any method for determining cAMP concentration. Typically, adenyl cyclase assays will be performed separately from binding assays, but it may also be possible to perform 5 binding and adenyl cyclase assays on a single preparation of cells.

Activation of receptor may also be determined based upon a measurement of intracellular calcium concentration. For example, transformed HEK-293 cells may be grown on glass cover slides to confluence. After rinsing, they may be incubated in the presence of an agent such as Fluo-3 or FURA-2 AM (Molecular Probe F-1221). After rinsing and further 10 incubation, calcium displacement may be measured using a photometer. Other types of assays for determining intracellular calcium concentrations are well known in the art and may also be employed.

Assays that measure the intrinsic activity of the receptor, such as those based upon inositol phosphate measurement, may be used in order to determine the activity of inverse 15 agonists. Unlike antagonists which block the activity of agonists but produce no activity of their own, inverse agonists produce a biological response diametrically opposed to the response produced by an agonist. For example, if an agonist promoted an increase in intracellular calcium, an inverse agonist would decrease intracellular calcium levels.

The radioligand and cell activation assays discussed above merely provide examples 20 of the types of assays that can be used for determining whether a particular test compound alters the binding of MCH to the SLC-1 receptor and acts as an agonist or antagonist. There are many variations on these assays that are compatible with the present invention. Such assays may involve the use of labeled antibodies as a means for detecting MCH that has bound to receptor or may take the form of the fluorescent imaging plate reader assays 25 described in the Examples section herein.

Examples

I. Methods

Preparation of Clone 1-18

A PCR-based strategy was used to clone the rat 1-18 gene (SLC-1). Rat spinal cord 30 mRNA was isolated using the FastTrack® kit (InVitrogen, San Diego, Ca). The templates for

PCR amplification were synthesized using GeneAmp RNA PCR kits (N808-0017 Perkin Elmer) with 200 ng of the rat spinal dorsal horn polyA+ RNA and were amplified using the following primers:

- TM3-5: 5'-G(C or T)G(A or C)(C or G)(A or G)(C or G)(C or T)ITIGA(C or T)
5 CGCTA-3' (SEQ ID NO:2)
- TM7-5: 5'-AAGC(C or T)(A or G)TA(G or T)AI(A or C or G)AI(A or C)GG(A or
G)TT-3' (SEQ ID NO:3).

The reaction mixture contained 200 pmoles of each of the TM3-5 and TM7-5 primers and 2.5 units of Taq DNA polymerase in 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris(HCl), 200 mM dNTPs, pH 9.0. The reaction tubes were heated at 95 °C for one minute and subjected to 39 cycles of denaturation (95 °C / 1 min), annealing (42 °C / 1 min) and extension (72 °C / 1 min). The amplified fragments were analyzed and size fractionated on a 1% agarose gel. Fragments between 500 bp and 800 bp were excised from the gel, purified using the Sephadex BandPrep™ kit from Pharmacia (cat# 27-9285-01), and subcloned into the pGEM-T vector from Promega (cat# A3600). Recombinant pGEM-T clones were selected randomly and plasmid DNA was prepared using the alkaline lysis method starting with 2 ml of bacterial culture. The Sanger dideoxy nucleotide chain termination method was used to sequence the DNA from these clones, with the T7 sequencing kit from Pharmacia (cat# 27-1682-01). The insert DNA fragment of the clone pGEMT-1-18 was excised from the vector using Pst I and Sac II, isolated from an agarose gel and labeled with ³²P by random primed synthesis using the Ready-To-Go™ DNA labeling kit (cat#27-9251-01)from Pharmacia. This probe was used to screen a rat brain stem-spinal cord cDNA library in λ ZAP II (Stratagene, cat# 936521). The filters were incubated with the probe for 18 hours at 65°C in 2x SSC, 5x Denhardt's solution and 0.2% SDS. The filters were rinsed twice in 0.1x SSC, 0.2% SDS at room temperature. 25 The filters were then washed twice for 45 min in 0.1x SSC, 0.2% SDS at 65°C, once for 45 min at 65°C in 5 mM EDTA, 0.2% SDS, pH 8.0 and finally rinsed with 0.1x SSC at room temperature.

Hybridization-positive phages were purified and their inserts rescued by helper phage mediated excision to yield plasmid DNA. The insert of plasmid pBS/1-18 was sequenced progressively with the 1-18-specific primers. To generate a mammalian expression vector, a 2 Kb Sma I - Xho I fragment from pBS/1-18 was isolated and subcloned into the Eco RV and

Xho I sites of pcDNA3 (InVitrogen, San Diego, Ca). This expression vector was called pcDNA3-1-18. Plasmid DNA was prepared using the Qiaprep system from Qiagen.

Expression

HEK-293 cells were transfected with a mammalian expression construct coding for the 1-
5 18 clone (pcDNA 3.0 vector, Invitrogen) using the Superfect reagent (Qiagen). A stable
receptor pool of 1-18 was developed by applying a selection marker (G418, 0.6 mg/ml) and
the cells were maintained in this selection medium. The presence of mRNA specific for clone
1-18 was assessed by Northern blot analysis and by the reverse transcriptase polymerase chain
reaction (RT-PCR).

10 *Ligands*

In order to identify the ligand of clone 1-18, a collection of peptide and non-peptide
ligands was obtained from commercial sources (Sigma, CalBiochem, American Peptide
Company, Bachem, RBI). The compounds were dissolved in water/DMSO at 30 iM and
placed in 96 well microplates. A total of 846 compounds (peptides and non-peptides) were
15 prepared and tested.

Assay

A functional assay was performed with FLIPR (Fluorescent Imaging Plate Reader,
Molecular Devices) using the fluorescent calcium indicator Fluo-3 (Molecular Probes) on a 96
well platform. HEK-293 cells, either expressing the receptor or wild type cells, were loaded
20 with Fluo-3 as follows. Stable HEK-293 clones expressing 1-18 or parental cells were plated
at a density of 70,000 cells/well in a 96 well plate. On the day of the experiment, the 1-18
cells were loaded with fluorescent solution (Dulbecco's modified medium with 10% fetal
bovine serum containing 4 ified medium with 10% fetal bovine serum containing 4 Probes)
on a 96 well platform. HEK-293 cells, either expressing the receptor or wild type cells, were
25 loaded with Fluo-3 as follows. Stable HEK-2 BSA (pH 7.4). The cells were analyzed using
the FLIPR system to measure the mobilization of intracellular calcium in response to different
compounds.

II. Results

HEK-293 cells endogenously express some GPCRs such as bradykinin receptors which
30 can be used as an internal control for assays. The background signal was established with all

of the compounds in the parental HEK-293 cells (non-transfected) using the FLIPR assay. HEK-293 cells expressing the clone 1-18 were stimulated with all compounds and calcium responses were compared with those in parental HEK-293 cells. Only one compound, melanin concentrating hormone (MCH), consistently elicited signals in the transformed cells but not the wild type cells. This indicates that MCH is interacting with the recombinantly expressed receptor. Confirmation of this conclusion was obtained by the observation of a dose-response relationship with MCH in the cells transfected with 1-18, but not in the non-transfected cells or in cells transfected with several other different receptors. Thus, it has been established that clone 1-18 is, in fact, a specific receptor for MCH and that this receptor can be used to screen 10 compounds which either mimic the action of MCH (agonists) or antagonize the action of MCH (antagonists).

Screening assays can be performed using the FLIPR assay described above. Alternatively, MCH can be iodinated and used as a tracer in radioligand binding assays on whole cells or membranes. Other assays that can be used include the GTP α S assay, adenylate cyclase 15 assays, assays measuring inositol phosphates, and reporter gene assays (e.g., those utilizing luciferase, aqueorin, alkaline phosphatase, etc.).

All references cited herein are fully incorporated by reference. Having now fully 20 described the invention, it will be understood by those of skill in the art that the invention may be performed within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.

CLAIMS

1. A method of assaying a test compound for its ability to bind to the SLC-1 receptor, comprising:
 - a) incubating a cell expressing the SLC-1 receptor gene with melanin concentrating hormone (MCH) and said test compound; and
 - b) determining the extent to which the binding of said MCH is displaced by said test compound.
2. The method of claim 1, wherein said cell expressing SLC-1 is a recombinant cell that has been transformed with a heterologous SLC-1 gene.
- 10 3. The method of claim 1, wherein said assay is a radioligand assay and said MCH or said test compound is radioactively labeled.
4. The method of claim 1, wherein said assay is an enzyme-linked immunosorbent assay (ELISA) and either said MCH or said test compound is joined to an enzyme.
- 15 5. The method of any one of claims 1-4, further comprising determining whether said test compound significantly increases or decreases either the adenyl cyclase or intracellular calcium concentration of said cell.
6. A method of determining if a test compound is an agonist, antagonist or inverse agonist of MCH, comprising:
 - a) incubating a cell expressing SLC-1 with said test compound;
 - 20 b) determining the intracellular adenyl cyclase activity or intracellular concentration of calcium of said cell during the incubation of step a);
 - c) comparing the results obtained in step b) with the results obtained when incubations are performed in the absence of said test compound; and
 - d) concluding that said test compound is an agonist of MCH if the level of adenyl cyclase
- 25 activity or intracellular calcium is significantly higher in the presence of said test compound than in its absence, or concluding that said test compound is an antagonist of MCH if the level of adenyl cyclase activity or intracellular calcium concentration is significantly lower in the presence of said test compound than in its absence.

7. The method of claim 6, wherein said cell is a recombinant cell that has been transformed with a heterologous SLC-1 gene.
8. The method of either claim 6 or claim 7, wherein said cell expressing SLC-1 and test compound are incubated in a medium further comprising MCH.

SEQUENCE LISTING

<110> AstraZeneca AB
Lembo, Paola
Cao, Jack
5 Walker, Philippe
Grazzini, Eric
Ahmad, Sultan

10 <120> Assays for Agonists, Antagonists and Inverse Agonists
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INTERNATIONAL SEARCH REPORT

International application No. PCT/SE 00/01010
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A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/567, C12Q 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, CONFERENCE PAPERS INDEX (CPI).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 0040725 A1 (TAKEDA CHEMICAL INDUSTRIES, LTD.), 13 July 2000 (13.07.00), abstract --	1-8
P,X	Nature, Volume 400, No 6741, July 1999, Chambers J. et al, "Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1", page 261 - page 265, see abstract and method in page 264 --	1-8
P,X	FEBS Lett, Volume 457, No 3, Sept 1999, Bachner D. et al, "Identification of melanin concentratinghormone (MCH) as the natu ral ligand for the orphan somatostatin-like receptor 1 (SLC-1).", page 522 - page 524, abstract --	1-8

 Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents
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- "E" earlier document but published on or after the international filing date
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- "P" document published prior to the international filing date but later than the priority date claimed
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- "&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/01010

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	Nature, Volume 400, No 6471, July 1999, Saito Y. et al, "Molecular characterization of the melanin-concentrating-hormone receptor" page 265 - page 269 --	1-8
X	FEBS Lett, Volume 398, No 2-3, December 1996, Kolakowski LF Jr. et al, "Characterization of a human gene related to genes encoding somatostatin receptors.", page 253 - page 258, abstract --	1-8
X	WO 9618651 A1 (SMITHKLINE BEECHAM CORPORATION), 20 June 1996 (20.06.96) --	1-8
A	US 5849708 A (ELFATHERIA MARATOS-FLIER), 15 December 1998 (15.12.98) --	1-8
A	WO 9639162 A1 (JOSLIN DIABETES CENTER, INC.), 12 December 1996 (12.12.96) -----	1-8

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SE 00/01010

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 0040725 A1	13/07/00	NONE		

WO 9618651 A1	20/06/96	AU 4522796 A EP 0871669 A JP 10511936 T ZA 9510686 A		03/07/96 21/10/98 17/11/98 03/07/96

US 5849708 A	15/12/98	AU 6041896 A CA 2223153 A EP 0871463 A JP 11507517 T WO 9639162 A		24/12/96 12/12/96 21/10/98 06/07/99 12/12/96

WO 9639162 A1	12/12/96	AU 6041896 A CA 2223153 A EP 0871463 A JP 11507517 T US 5849708 A		24/12/96 12/12/96 21/10/98 06/07/99 15/12/98

